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Characterization of a Bartonella Bacilliformis Human Factor H-Binding Protein

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Abstract –

Bartonella bacilliformis is a human bacterial pathogen and the etiological agent of Carrión's Disease. *B. bacilliformis* is serum-resistant, allowing it to survive in the human bloodstream and persist and replicate in erythrocytes. Human factor H is a circulating protein in human blood that is part of the complement cascade of innate immune defense. Factor H binds to self-cells and prevents auto-immunity by complement fixation. A Far-Western blot followed by mass spectrometry analysis suggests that several *B. bacilliformis* proteins can bind to human Factor H. Here, we describe one protein, BB1133; an outer membrane auto-transporter and the Factor H-binding protein identified by mass spectrometry. Two distinct domains of BB1133 were cloned and expressed in *E. coli* using the Gateway cloning system and pET-DEST42 vector. IPTG induction of the constructs, followed by a Far-Western blot with human Factor H as a probe, shows that the passenger domain of BB1133 is responsible for binding human factor H.

Background –

- *B. bacilliformis* is the etiological agent of Carrión's disease, a vector-borne disease transmitted to humans via phlebotomine sand flies.
- Bb1133 is a trimeric autotransporter adhesin (TAA) protein. TAA's are Gram-negative outer membrane (OM) proteins [1], secreted by the bacterium, and are comprised of two parts: the autotransporter and the passenger domain. The passenger domain is the portion of TAAs responsible for binding host ligands.
- Factor H (fH) is a part of the complement cascade of the innate human immune system. fH binds to self-cells and prevents them from being destroyed by immune activities of complement fixation.
- *B. bacilliformis* binds human fH, and we hypothesize that this allows the pathogen to resist complement and persist in the blood stream.

Aims -

- Identify Bb1133 autotransporter (AUTO) and passenger (PD) domains (**Fig. 3**)
- Clone Bb1133 AUTO and PD into *E. Coli* pEXP42 plasmid vectors (**Fig. 4**)
- Verify His-tag expression in pEXP42 AUTO and PD via a HisHRP Far-Western (FW) blots (**Fig. 5**)
- Identify whether AUTO or PD are fH-binding via fH FW blots (**Fig. 6**)

Fig. 1: SDS-PAGE (left) and corresponding FW blot (right) showing four *B. bacilliformis* membrane proteins that bind human fH (far right). MALDI-TOF MS was used to identify the arrowed protein as Bb1133.

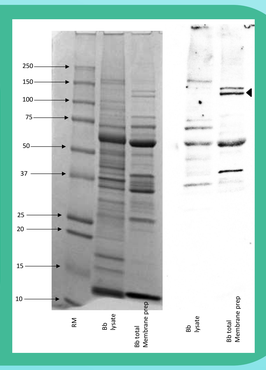
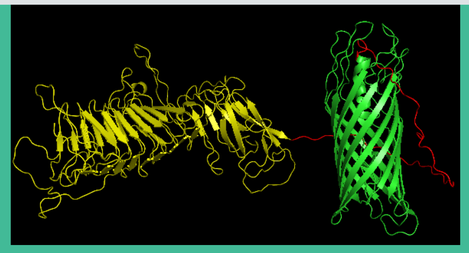


Fig. 2: Phyre2 3D Protein Model of Bb1133. The yellow section is the predicted passenger domain (PD), the green portion is the autotransporter (AUTO), and the red filament is the stalk. (Adapted from [2])



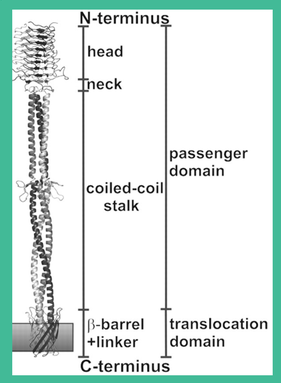


Fig. 3: Trimeric Autotransporter Adhesin (TAA) Domains. Typical domains of TAAs are shown. AUTO and PD portions of Bb1133 were subcloned since intact Bb1133 was toxic to *E. coli*. (Figure modified from [1]).

Fig. 4: Plasmid Maps of Subclones. A) Bb1133 PD cloned into pEXP42 plasmid. B) Bb1133 AUTO cloned into pEXP42 plasmid.

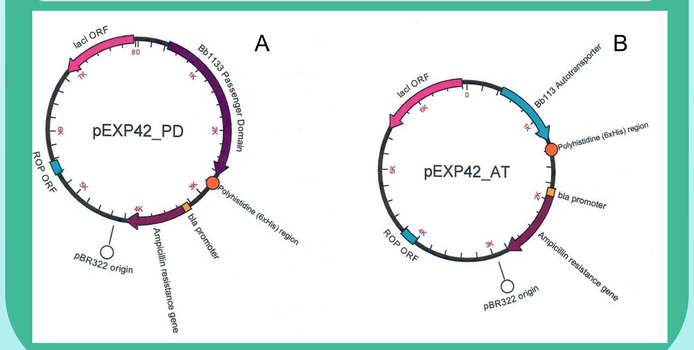


Fig. 5: HisHRP (Nickel-Activated Horseradish Peroxidase) FW Blot. Blot (with corresponding Coomassie SDS-PAGE gel on left) indicates that the His-tag is detectable in both pEXP42AUTO and pEXP42PD. Both recombinant proteins are in the insoluble fraction of the bacterial cell. The PD was broken into several fragments with His-tags (lane 7).

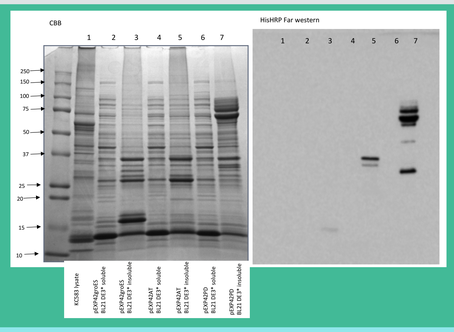
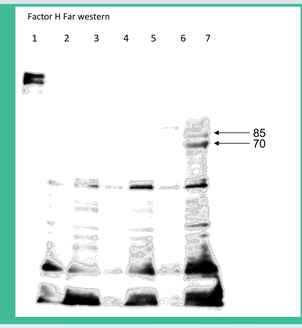


Fig. 6: FW Blot showing that the PD of Bb1133 is responsible for binding human fH. Recombinant PD bands of 70 and 85 kDa bind fH. [The 85-kDa band is the full-length PD of Bb1133]. The FW blot corresponds to the Coomassie blue SDS-PAGE gel shown in Fig. 5.



Results and Discussion –

- Subcloning of two Bb1133 domains into pEXP42, expression in *E. coli* and FW blots demonstrate that the PD of Bb1133 is responsible for binding human fH and is therefore likely used by the pathogen to avoid destruction by human complement during infection.

Future Directions –

- Knockout Bb1133 in *Bartonella bacilliformis.*
- Check for fH binding using FW blots in mutated *Bartonella bacilliformis*
- Determine whether complement resistance is impaired in mutant versus wild-type strains.

References –

1. Andrzej Łyskowski, Jack C. Leo, Adrian Goldman. 2011. "Structure and Biology of Trimeric Autotransporter Adhesins". *Advances in Experimental Medicine and Biology*. 715:143.

2. Kelley LA *et al.* 2015. *Nature Protocols* 10:845-858.

3. Kornelia M. Mikula, *et. al.* 2012. "The Translocation Domain in Trimeric Autotransporter Adhesins Is Necessary and Sufficient for Trimerization and Autotransportation". *Journal of Bacteriology*. 194: 827-838.

Acknowledgments -

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